

## CLAIMS

We claim:

1. An isolated polynucleotide molecule selected  
5 from the group consisting of:

(a) DNA molecules encoding a Zins2 testis-specific insulin homolog polypeptide and comprising a nucleotide sequence as shown in SEQ ID NO: 1 from nucleotide 1 to nucleotide 564;

10 (b) allelic variants of (a);

(c) DNA molecules encoding a Zins2 testis-specific insulin homolog polypeptide which are least 85% identical in nucleotide sequence to (a) or (b); and

15 (d) degenerate nucleotide sequences encoding a Zins2 testis-specific insulin homolog polypeptide.

2. An expression vector comprising the following operably linked elements: a transcriptional promoter; a DNA segment selected from the group consisting of:

20 (a) DNA molecules encoding a Zins2 testis-specific insulin homolog polypeptide and comprising a nucleotide sequence as shown in SEQ ID NO: 1 from nucleotide 1 to nucleotide 564;

(b) allelic variants of (a);

25 (c) DNA molecules encoding a Zins2 testis-specific insulin homolog polypeptide and which are least 85% identical in nucleotide sequence to (a) or (b);

and a transcriptional terminator.

30 3. A cultured cell into which has been introduced an expression vector comprising the following operably linked elements:

a transcriptional promoter;

a DNA segment selected from the group consisting of:

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(a) DNA molecules encoding a testis-specific insulin homolog polypeptide and comprising a nucleotide sequence as shown in SEQ ID NO: 1 from nucleotide 1 to nucleotide 564;

(b) allelic variants of (a);

5 (c) DNA molecules encoding a testis-specific insulin homolog polypeptide which are least 85% identical in nucleotide sequence to (a) or (b); and

a transcriptional terminator, and wherein the cell expresses a testis-specific insulin homolog polypeptide  
10 encoded by the DNA segment.

4. A method for producing a Zins2 testis-specific insulin homolog polypeptide comprising culturing a cell into which has been introduced an expression vector comprising the  
15 following operably linked elements: a transcriptional promoter; a DNA segment selected from the group consisting of:

(a) DNA molecules encoding a Zins2 testis-specific insulin homolog polypeptide and comprising a nucleotide sequence as shown in SEQ ID NO: 1 from nucleotide 1 to  
20 nucleotide 564;

(b) allelic variants of (a);

(c) DNA molecules encoding a Zins2 testis-specific insulin homolog polypeptide which are least 85% identical in nucleotide sequence to (a) or (b); and

25 a transcriptional terminator,  
whereby the cell expresses a Zins2 testis-specific insulin homolog polypeptide encoded by the DNA segment and recovering the Zins2 testis-specific insulin homolog.

30 5. A method for producing a Zins2 testis-specific insulin homolog polypeptide according to Claim 4 wherein said cell further comprises a second expression vector comprising the following operably linked elements:

a transcriptional promoter;  
35 a DNA sequence encoding a prohormone convertase; and  
a transcriptional terminator.

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6. A method for producing a Zins2 testis-specific insulin homolog polypeptide according to Claim 5 wherein said prohormone convertase is selected from the group consisting of  
5   prohormone convertase 2, prohormone convertase 3, prohormone convertase 4 and furin.

7. A post-translationally modified Zins2 testis-specific insulin homolog polypeptide or polypeptide fragment  
10   having the amino acid sequence from amino acid residue 53 to amino acid residue 162 of SEQ ID NO:2; the amino acid sequence from amino acid residue 55 to amino acid residue 172 of SEQ ID NO:13 or the amino acid sequence from amino acid residue 201 to amino acid residue 213 of SEQ ID NO:13.

8. An antibody that specifically binds to an epitope of a Zins2 testis-specific insulin homolog polypeptide selected from the group consisting of:

(a) polypeptides comprising a sequence of amino  
20   acids encoded by the nucleotide sequence as shown in SEQ ID NO: 1;

(b) allelic variants of (a); and

(c) testis-specific insulin homolog polypeptides which are at least 85% identical to (a), (b) or (c).

9. A binding protein that specifically binds to an epitope of an isolated Zins2 testis-specific insulin homolog polypeptide selected from the group consisting of:

(a) polypeptides comprising a sequence of amino  
30   acids encoded by the nucleotide sequence as shown in SEQ ID NO: 1;

(b) allelic variants of (a); and

(c) testis-specific insulin homolog polypeptides which are at least 85% identical to (a), (b) or (c).

10. A binding protein that specifically binds to an epitope of a polypeptide selected from the group consisting of:

(a) polypeptides comprising a sequence of amino acids encoded by the nucleotide sequence as shown in SEQ ID NO:12;

(b) allelic variants of (a); and

(c) testis-specific insulin homolog polypeptides which are at least 85% identical to (a) or (b).

11. A method of enhancing viability of cryopreserved sperm for use in fertilization of an egg, wherein a Zins2 testis-specific insulin homolog polypeptide selected from the group consisting of:

(a) polypeptides comprising a sequence of amino acids encoded by the nucleotide sequence as shown in SEQ ID NO: 1 or SEQ ID NO:12;

(b) allelic variants of (a); and

(c) Zins2 testis-specific insulin homolog polypeptides which are at least 85% identical to (a) or (b);

is added to sperm, an egg, an egg-sperm mixture, prior to fertilization.

12. A method of enhancing sperm motility wherein a Zins2 testis-specific insulin homolog polypeptide selected from the group consisting of:

(a) polypeptides comprising a sequence of amino acids encoded by the nucleotide sequence as shown in SEQ ID NO: 1 or SEQ ID NO:12;

(b) allelic variants of (a); and

(c) Zins2 testis-specific insulin homolog polypeptides which are at least 85% identical to (a) or (b);

is added to sperm, an egg, an egg-sperm mixture, prior to fertilization.

13. A method of enhancing sperm motility according to Claim 12 wherein said Zins2 testis-specific insulin homolog is added to sperm following cryopreservation.

5 14. A method of enhancing an egg-sperm interaction wherein a Zins2 testis-specific insulin homolog polypeptide selected from the group consisting of:

(a) polypeptides comprising a sequence of amino acids encoded by the nucleotide sequence as shown in SEQ ID  
10 NO: 1 or SEQ ID NO:12;

(b) allelic variants of (a); and

(d) Zins2 testis-specific insulin homolog polypeptides which are at least 85% identical to (a) or (b);

15 is added to sperm, an egg, an egg-sperm mixture prior to fertilization.

15. A method of enhancing fertilization during assisted reproduction wherein a Zins2 testis-specific insulin homolog polypeptide selected from the group consisting of:

20 (a) polypeptides comprising a sequence of amino acids encoded by the nucleotide sequence as shown in SEQ ID NO: 1 or SEQ ID NO:12;

(b) allelic variants of (a); and

25 (c) Zins2 testis-specific insulin homolog polypeptides which are at least 85% identical to (a) or (b);

is combined with sperm, an egg, an egg-sperm mixture prior to fertilization of the egg.

30 16. A method of enhancing fertilization according to Claim 15 wherein said assisted reproduction is artificial insemination.

35 17. A method of enhancing fertilization according to Claim 15 wherein said assisted reproduction is *in vitro* fertilization.

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18. A method of contraception wherein an antagonist of a Zins2 testis-specific insulin homolog polypeptide selected from the group consisting of:

(a) polypeptides comprising a sequence of amino acids encoded by the nucleotide sequence as shown in SEQ ID NO: 1 or SEQ ID NO:12;

(b) allelic variants of (a); and

(c) Zins2 testis-specific insulin homolog polypeptides which are at least 85% identical to (a) or (b);

is administered to a recipient to prevent fertilization of an egg.

19. A method of contraception according to Claim 18 wherein said antagonist is an anti-Zins2 binding protein.

20. A method of immunocontraception wherein a vaccine containing a Zins2 testis-specific insulin homolog polypeptide selected from the group consisting of:

(a) polypeptides comprising a sequence of amino acids encoded by the nucleotide sequence as shown in SEQ ID NO: 1 or SEQ ID NO:12;

(b) allelic variants of (a); and

(c) Zins2 testis-specific insulin homolog polypeptides which are at least 85% identical to (a) or (b);

is administered to a recipient to prevent fertilization of an egg.

21. An isolated, mature rat Zins2 testis-specific insulin homolog protein consisting of:

a B chain having the amino acid sequence from amino acid residue 24 to amino acid residue 51 of SEQ ID NO:2;

an A chain having the amino acid sequence from amino acid residue 163 to amino acid residue 188 of SEQ ID NO:2,

wherein the B chain and A chain are joined by inter- and intra-chain disulfide bonds.

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22. An isolated, mature rat Zins2 testis-specific insulin homolog protein according to claim 21, wherein said protein consists of:

5 a B chain having the amino acid sequence from amino acid residue 24 to amino acid residue 52 of SEQ ID NO:2;

an A chain having the amino acid sequence from amino acid residue 163 to amino acid residue 188 of SEQ ID NO:2,

10 wherein the B chain and A chain are joined by inter- and intra-chain disulfide bonds.

23. An isolated, mature human Zins2 testis-specific insulin homolog protein consisting of:

15 a B chain comprising amino acid residue 23 to amino acid residue 53 of SEQ ID NO:13;

an A chain comprising amino acid residue 173 to amino acid residue 198 of SEQ ID NO:13;

20 wherein the B chain and A chain are joined by inter- and intra-chain disulfide bonds.

24. An isolated, mature human Zins2 testis-specific insulin homolog protein according to claim 23, wherein said protein consisting of:

25 a B chain comprising amino acid residue 23 to amino acid residue 54 of SEQ ID NO:13;

an A chain comprising amino acid residue 173 to amino acid residue 198 of SEQ ID NO:13;

30 wherein the B chain and A chain are joined by inter- and intra-chain disulfide bonds.

25. An isolated, mature human Zins2 testis-specific insulin homolog protein according to claim 23, wherein said protein consisting of:

35 a B chain comprising amino acid residue 23 to amino acid residue 54 of SEQ ID NO:13;

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an A chain comprising amino acid residue 173 to amino acid residue 199 of SEQ ID NO:13;

wherein the B chain and A chain are joined by inter- and intra-chain disulfide bonds.

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26. An isolated, mature human Zins2 testis-specific insulin homolog protein according to claim 23, wherein said protein consisting of:

a B chain comprising amino acid residue 23 to amino acid residue 54 of SEQ ID NO:13;

an A chain comprising amino acid residue 173 to amino acid residue 200 of SEQ ID NO:13;

wherein the B chain and A chain are joined by inter- and intra-chain disulfide bonds.

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27. A method for detecting Zins2 post-translationally modified polypeptides or polypeptide fragments comprising:

exposing a solution possibly containing Zins2 post-translationally modified polypeptides or polypeptide fragments to an antibody attached to a solid support, wherein said antibody binds to a first epitope of a Zins2 post-translationally modified polypeptide or polypeptide fragment;

washing said immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a Zins2 post-translationally modified polypeptide or polypeptide fragment, wherein the second antibody is associated with a detectable label; and

detecting the detectable label. Levels of Zins2 post-translationally modified polypeptides or polypeptide fragments in serum or biopsy are then correlated to tumor activity.

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28. A method according to claim 27, wherein said Zins2 post-translationally modified polypeptides or

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polypeptide fragments are selected from the group consisting of:

5 a) a polypeptide consisting of the amino acid sequence from amino acid residue 53 to amino acid residue 162 of SEQ ID NO:2;

b) a polypeptide consisting of the amino acid sequence from amino acid residue 55 to amino acid residue 172 of SEQ ID NO:13;

10 c) a polypeptide consisting of the amino acid sequence from amino acid residue 201 to amino acid residue 213 of SEQ ID NO:13; and

d) polypeptide fragments of a, b, or c.

15 29. A method for detecting nucleic acid sequences encoding Zins2 post-translationally modified polypeptides or polypeptide fragments comprising:

20 incubating a single-stranded probe molecule with RNA isolated from a biological sample under conditions of temperature and ionic strength that promote base pairing between the probe and target Zins2 RNA species;

separating unbound probe from hybridized molecules; and

detecting the presence of probe-Zins2 RNA hybrids.

25 30. A method according to claim 29, wherein said single-stranded probe molecule is at least 5 consecutive nucleotides selected from the group consisting of:

30 a) nucleotides 157-487 of SEQ ID NO:1;  
b) nucleotides 179-532 of SEQ ID NO:12; and  
c) nucleotides 617-655 of SEQ ID NO:12.